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Charlotte Schoelinck, Corinne Cruaud, Jean-Lou Justine. Are all species of *Pseudorhabdosynochus* strictly host specific? - a molecular study. *Parasitology International*, 2012, 61, 10.1016/j.parint.2012.01.009. 10.1016/j.parint.2012.01.009 . mnhn-00673113

**HAL Id: mnhn-00673113**

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Submitted on 22 Feb 2012

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## Are all species of *Pseudorhabdosynochus* strictly host specific? – a molecular study

Charlotte Schoelinck (a, b) Corinne Cruaud (c), Jean-Lou Justine (a)

(a) UMR 7138 Systématique, Adaptation, Évolution, Muséum National d'Histoire Naturelle, Département Systématique et Évolution, CP 51, 55 Rue Buffon, 75231 Paris cedex 05, France.

(b) Service de Systématique moléculaire (CNRS-MNHN, UMS2700), Muséum National d'Histoire Naturelle, Département Systématique et Évolution, CP 26, 43 Rue Cuvier, 75231 Paris Cedex 05, France.

(c) Génoscope, Centre National de Séquençage, 2 rue Gaston Crémieux, CP 5706, 91057 Évry Cedex, France.

e-mails: CS : schoelinck@mnhn.fr

JLJ: justine@mnhn.fr

CC: cruaud@genoscope.cns.fr

Corresponding author:

Charlotte Schoelinck, Service de Systématique moléculaire, Muséum National d'Histoire Naturelle, Département Systématique et Évolution, CP 26, 43 Rue Cuvier, 75231 Paris Cedex 05, France, Telephone : +33 1 40 79 31 65, Fax : +33 1 40 79 38 44

## Abstract

Species of the diplectanid monogenean genus *Pseudorhabdosynochus* are strictly host-specific (specialist), with the exception of *P. cyanopodus*, which was reported in New Caledonia, South Pacific, from two host species, *Epinephelus cyanopodus* and *E. chlorostigma*. We sequenced the COI gene of both host fish species and of their monogeneans. Morphological identification and pairwise distances showed that the two fish species were distinct (difference 6.1–6.6%), but that their monogeneans were not (difference 0–1.5%). A morphological study of sclerotised parts showed that specimens of *P. cyanopodus* are similar in both fish. Most species of groupers and their associated *Pseudorhabdosynochus* species are from warm surface waters, but the two groupers *E. cyanopodus* and *E. chlorostigma* are usually caught in deep-sea on the outer slope of the coral reef. This suggests that acquisition of a less strict host specificity is an adaptation of *P. cyanopodus* to deep-sea hosts.

## 1. Introduction

Species of the monogenean diplectanid genus *Pseudorhabdosynochus* are strictly host-specific. A study of more than 25 species of groupers [1] in the natural environment off New Caledonia, South Pacific, demonstrated that most grouper species harboured 1–8 species of *Pseudorhabdosynochus*, and that each of these monogenean species was “specialist”, i.e. found on a single host species [1-9]. An exception was *P. cyanopodus* Sigura & Justine, 2008, reported both from its type-host, *Epinephelus cyanopodus*, and from *E. chlorostigma*; however, this report was based only on comparative measurements [8]. In this paper, we provide additional morphological evidence and the first molecular confirmation of the presence of the same *Pseudorhabdosynochus* species on these two species of hosts, and we also confirm, with molecular methods, that the two host species are distinct.

## 2. Material and Methods

Fish were collected from off the barrier reef of New Caledonia [5,8] or from the fish market of Nouméa, New Caledonia. Specimens of *E. chlorostigma* and *E. cyanopodus* were morphologically identified according to keys [10] and their identification was confirmed from photographs by confirmed ichthyologists. A photograph of specimen JNC3142 of *E. chlorostigma* was deposited in FishBase ([www.fishbase.org](http://www.fishbase.org)). Parasites were collected [8] from 2 specimens of each fish species. Monogeneans were either processed for morphological examination [8] or for molecular characterization; in the latter case, each monogenean was examined with a microscope for species identification and then destroyed in the process of DNA extraction.

Fish DNA was extracted from tissue samples using NucleoSpin 96 tissue kit (Macherey-Nagel, Düren, Germany). Approximately 654 bp were amplified from the 5' region of the Cytochrome Oxidase I (COI) gene from mitochondrial DNA using FishF1 (forward 5'-

TCAACCAACCACAAAGACATTGGCAC-3') and FishR1 (Reverse 5'-TAGACTTCTGGGTGGCCAAAGAATCA3') [11]. Monogenean DNA was extracted from the whole specimen using DNeasy® 96 Blood & Tissue kit (Qiagen). A fragment of 424 bp of COI gene was amplified using the specific primers COI-ASmit1 (forward 5'-TTTTTTGGGCATCCTGAGGTTTAT-3') and COI-ASmit2 (reverse 5'-TAAAGAAAGAACATAATGAAAATG-3') [12]. Each PCR reaction was performed in final 20 µl volume containing 1 ng of DNA, 1 × reaction buffer, 0.26 mM dNTP, 0.8 µM of each primer, 5% DMSO and 1.5 units of Taq DNA polymerase (Qiagen). Thermocycles consisted of an initial denaturation step at 94°C for 2', followed by 37 cycles of denaturation at 94°C for 30'', annealing at 48°C for 40'' and extension at 72°C for 1'. The final extension was conducted at 72°C for 10'. Purification and cycle-sequencing reactions were performed at the Génoscope (Évry, France), using the BigDye Terminator version 3 sequencing kit, the GeneAmp PCR System 9700 and a capillary ABI3730 DNA Analyser, all from Applied Biosystems. Both DNA strands were sequenced for all PCR products. Sequences were edited and assembled using Sequencher 4.9 (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences for this study were deposited as GenBank ID: JQ412500–JQ412503 (fish) and JQ400132–JQ400138 (monogeneans).

COI sequences were automatically aligned using ClustalW implemented in BioEdit version 7.0.5.3 [13]. The accuracy of automatic alignments was confirmed by eye. Uncorrected pairwise distances were computed using Mega 5 [14]. Results were visualized on a pairwise difference matrix. The identity of fish species was checked using BLAST in BOLD [15]; issues in the specific attribution of sequences are outlined below (3.1).

### 3. Results

#### 3.1. Analysis of fish COI sequences

Sequences of our specimens of *E. cyanopodus* and *E. chlorostigma* showed low intraspecific variability (respectively 0.2% and 0.5%), and high interspecific differences (6.1–6.6 %) (Table 1). COI sequences are considered reliable indicators for species delimitation [11,16], and thus we consider that the two fish species were distinct. Sequences of *E. cyanopodus* from our specimens matched with the three deposited sequences of this species in BOLD with 99.84–100% similarity (date: 11 January 2012; unpublished sequences in BOLD AMS513-08, EPINE003-11, EPINE003-12; no sequence in GenBank); we thus consider that molecular evidence confirms morphological identification. Sequences of our specimens of *E. chlorostigma* matched with the six deposited sequences of this species in GenBank with only 95.04–95.37% (date: 11 January 2012; HQ149841 [17]; EF609514–5, EU39202–4 [18]). According to Heemstra & Randall [10], two species, *E. polylepis* and *E. gabriellae*, were often misidentified as *E. chlorostigma*, but have different geographical distributions. On the basis of colour pattern and biogeographical distribution, we suggest that sequences previously deposited in GenBank as *E. chlorostigma* from the west coast of India [18] and the Persian Gulf [17] correspond to

*E. polylepis* (this species ranges from the west coast of India to the Gulf of Aden [10]). We provisionally consider that our specimens correspond to *E. chlorostigma* (the species is already recorded from New Caledonia [10,19]) but we urge caution because the type locality of *E. chlorostigma* is the Seychelles in the Indian Ocean [10]; a molecular reappraisal of the status of *E. chlorostigma* and its close relatives is certainly needed, but is beyond the scope of this parasitological paper.

### 3.2. Morphology of monogeneans

Morphological examination of monogeneans (Fig. 1) demonstrated that the key structures which allow species differentiation [4,8,20], i.e. the sclerotised male copulatory organ (Fig. 1A, B) and vagina (Fig. 1C–E), were similar in *P. cyanopodus* from *E. chlorostigma* to the corresponding structures found in specimens from *E. cyanopodus* (see Figures 2–4 in [5]). The squamodiscs (Fig. 1J) showed “spurs”, a structure not mentioned in the original description of *P. cyanopodus* [5], but this is not a reliable systematic character [8]. Measurements were similar (see Tables 2, 3 in [8]).

### 3.3. Analysis of monogenean COI sequences

Seven monogenean sequences were obtained. The difference matrix of monogenean COI sequences (Table 2) showed that variability ranged 0–0.5% for specimens from *E. cyanopodus* and 0.7–1.5% for specimens from *E. chlorostigma*. Variability between monogeneans from the two fish species did not exceed 1.5%. We consider that such small variability demonstrates that monogenean specimens from both fish species belong to a single species [21–23].

## 4. Discussion

Host specificity is one of the basic problems of parasitology. Euzet & Combes [24] discussed the problem of species in parasites and remarked that most monogeneans are strictly host-specific (“oioxenous specificity”) but rejected the description of new species of parasites on the simple basis of their recovery from a new species of host. Several genera of monopisthocotylean monogeneans include many species: these include the gyrodactylid *Gyrodactylus* [25], the dactylogyrids *Anacanthorus* [26] and *Dactylogyrus* [27], the ancyrocephalids *Haliotrema* [28,29], *Haliotrematoides* [30] and *Ligophorus* [31], and the diplectanids *Lamellodiscus* [23,32–34] and *Pseudorhabdosynochus* [1–9,20,35–38]. Strict or non-strict host specificity does not follow a regular pattern within genera; if we limit examples to diplectanids, species of *Calydiscooides* [39–41] and *Lamellodiscus* [23,32–34] show either strict specificity or are found in a small number of host species, but species of *Pseudorhabdosynochus* have hitherto been considered strictly host-specific, or “specialists” [1–9]. Reports of species of *Pseudorhabdosynochus* found in two fish species were considered erroneous and attributed to insufficient morphological differentiation [20,42] or to the fact that living fish of different species were kept together in a tank, thus allowing accidental host changes [20]. In wild conditions in

New Caledonia, most species of *Pseudorhabdosynochus* have been found on a single host species only. The only exceptions are very small numbers of *P. duitoe* and *P. huitoe* [5,8], and *P. podocyanus*, and the subject of the present study, *P. cyanopodus*.

The present study is the first molecular and morphological demonstration of infection of two species of naturally infected hosts, *E. cyanopodus* and *E. chlorostigma*, with the same species of *Pseudorhabdosynochus*. Our morphological [8] and molecular (unpublished COI sequences) studies of the other species of *Pseudorhabdosynochus* found on the same hosts show that they are distinct (no molecular data for *P. podocyanus*, which is rare).

The question is thus what conditions led to this exception? Our study of COI genes showed that the two fish species are distinct, confirming an earlier phylogeny based on other genes [43]. Although the depth recorded for both species is variable (4–300 m for *E. chlorostigma*, 2–150 m for *E. cyanopodus* [10,19]), in New Caledonian waters adults of both species are deep-sea fish, usually caught off the barrier reef at depths of 60–200 m. Parasite diversity is known to be much smaller in the deep sea than in surface waters [44–47], especially in comparison to such high diversity spots as coral reefs [1,48], where most groupers live and from where most *Pseudorhabdosynochus* species have been described. In *Dactylogyrus* species from cyprinids in Europe, it has been demonstrated [49] that certain duplication events gave rise to generalist species (i.e. generalist parasite species are sometimes more derived than specialist species [49]). If such a process also occurs in *Pseudorhabdosynochus*, we may hypothesize that the low specificity of *P. cyanopodus* is a secondary adaptation to deep-sea conditions, where hosts are rare and separated by wide areas, and that infesting two species of hosts helps perpetuating the parasite species. Interestingly, *P. epinepheli*, another species recorded from *E. chlorostigma* [20] has a single host in New Caledonia but apparently several hosts in other localities [20,50,51]; however, molecular evidence is unavailable for *P. epinepheli*. We do not know why this process did not affect the other diplectanids [5,8] from *E. cyanopodus* and *E. chlorostigma*, or those from other deep-sea groupers studied in the same locality [52,53], all of which appear to be strictly specific species.

## Acknowledgements

The identification of the hosts was kindly confirmed (from photographs) by John E. Randall (Bishop Museum, Hawaii). Aude Sigura and Émilie Henry, students, participated in the parasitological survey. Claude Chauvet (UNC, New Caledonia) kindly provided fish. David Gibson (NHM, London) provided advice for the title. Rod Bray (NHM, London) kindly edited the English. Funded by ATM barcode (MNHN) and ATM Biodiversité actuelle et fossile (MNHN).

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## Tables

**Table 1.** Difference matrix of uncorrected pairwise differences between COI sequences of specimens of the two fish species *E. cyanopodus* and *E. chlorostigma* (two specimens per species). JNC numbers refer to individual fish identification.

Fish species and specimen	Sequence	[1]	[2]	[3]
<i>E. chlorostigma</i> JNC3141 [1]	JQ412501			
<i>E. chlorostigma</i> JNC3142 [2]	JQ412500	0.005		
<i>E. cyanopodus</i> JNC3262 [3]	JQ412503	0.066	0.063	
<i>E. cyanopodus</i> JNC1625 [4]	JQ412502	0.065	0.061	0.002

**Table 2.** Difference matrix of uncorrected pairwise differences between COI sequences of specimens of *P. cyanopodus* from two fish species. JNC numbers refer to individual fish identification.

Host fish species and specimen	Monogenean specimen	Sequence	[1]	[2]	[3]	[4]	[5]	[6]
<i>E. cyanopodus</i> JNC3262	[1]	JQ400132	0.000					
<i>E. cyanopodus</i> JNC3262	[2]	JQ400133	0.000					
<i>E. cyanopodus</i> JNC3262	[3]	JQ400134	0.000	0.000				
<i>E. cyanopodus</i> JNC3144	[4]	JQ400137	0.005	0.005	0.005			
<i>E. chlorostigma</i> JNC3141	[5]	JQ400135	0.007	0.007	0.007	0.002		
<i>E. chlorostigma</i> JNC3141	[6]	JQ400136	0.015	0.015	0.015	0.010	0.012	
<i>E. chlorostigma</i> JNC3142	[7]	JQ400138	0.009	0.010	0.009	0.005	0.007	0.015

## Figure Legends

**Fig. 1.** *Pseudorhabdosynochus cyanopodus* Sigura & Justine, 2008 from *Epinephelus chlorostigma*. A, B, quadriloculate male copulatory organ; C–D, sclerotised vagina; F–I, haptor hard parts (F, lateral bar; G, ventral hamulus; H, dorsal hamulus; I, ventral bar); J, dorsal squamodisc (dorsal view). J, carmine; A, C, D, F–I, picrate; B, D, Berlese.

